Cooperation between wild lactococcal strains for cheese aroma formation

F. Amárita, M. de la Plaza, P. Fernández de Palencia, T. Requena*, C. Peláez

Department of Dairy Science and Technology, Instituto del Frío (CSIC), José Antonio Novais 10. Ciudad Universitaria, 28040 Madrid, Spain

Running title: Lactococcal cooperation for cheese aroma

*Correspondence to: Teresa Requena. Instituto del Frío (CSIC), José Antonio Novais 10, Ciudad Universitaria, 28040 Madrid, Spain. Tel.: +34-915492300; Fax: +34-915493627; *E*-mail address: trequena@if.csic.es

Abstract

Several wild lactococcal strains were tested for their ability to produce aroma compounds during growth in milk. Strains were incubated alone and in combination with *Lactococcus lactis* IFPL730, which is characterized by showing α-keto acid decarboxylase activity. Volatile compounds from incubated milks were analyzed by means of solid-phase microextraction (SPME) and evaluated by gas chromatography-mass spectrometry (GC-MS). Incubated milks were also sniffed for sensory analysis to describe aroma attributes. The combination of *L. lactis* IFPL326 that showed the highest branched chain aminotransferase activity with IFPL730 contributed to the highest formation of leucine derived volatile compounds such as 3-methylbutanal, 3-methyl-1-butanol and 2-hydroxy-4-methyl pentanoic acid methyl ester. In addition, the milk incubated with this combination of strains was awarded by the test panellists with the highest scores for "ripened cheese" attribute and aroma intensity. The results indicate that combination of *L. lactis* strains harbouring complementary catabolic routes can contribute to improve cheese aroma formation, resulting the combined cultures with *L. lactis* IFPL730 in higher volatile compound formation than isolate strains.

Keywords: Lactococcus lactis; cheese aroma; amino acid catabolism

1. Introduction

Development of aroma in cheese is a complex process that, in addition, is perceived as one of the foremost reason for product acceptance by consumers. Several chemical and biochemical reactions lead to development of cheese aroma, among them amino acid catabolism by lactic acid bacteria (LAB) has been considered of paramount importance. LAB usually initiate amino acid catabolism by a transamination reaction that leads to the formation of α-keto acids that reversively convert into hydroxy acids (Yvon, Thirouin, Rijnen, Fromentier, & Gripon, 1997; Amárita, Requena, Taborda, Amigo, & Peláez, 2001a; Williams, Noble, & Banks, 2001). There are several LAB aminotransferases characterized in the literature. Aromatic aminotransferase from *Lactococcus lactis* (Yvon et al., 1997; Gao & Steele, 1998) converts aromatic amino acids, but also leucine and methionine. *L. lactis* branched-chain aminotransferase (Atiles, Dudley, & Steele, 2000; Yvon, Chambellon, Bolotin, & Roudot-Algaron, 2000) converts isoleucine, leucine and valine, and to a much lesser extent methionine, cysteine and phenylalanine. A third aminotransferase activity named aspartate aminotransferase that also uses α-ketoglutarate as amino group-acceptor has been described in *L. lactis* (Dudley & Steele, 2001). Recently, Thage, Rattray, Laustsen, Ardö, Barkholt and Houlberg (2004) characterized an aminotransferase from *Lactobacillus paracasei* that specifically converts branched-chain amino acids.

Amino acid transamination in cheeses is rather limited in the absence of exogenous added α-ketoglutarate, since this keto acid is essential for the amino acid transamination (Yvon, Berthelot, & Gripon, 1998; Banks, Yvon, Gripon, de la Fuente, Brechany, Williams, & Muir, 2001). An alternative to provide cells with extra α-ketoglutarate for amino acid transamination is its regeneration from glutamate by a glutamate

dehydrogenase recently detected in several LAB species (Tanous, Kieronczyk, Helinck, Chambellon, & Yvon, 2002; Kieronczyk, Skeie, Langsrud & Yvon, 2003). The α-keto acids are converted by LAB to carboxylic acids of impact in cheese aroma, such as isovaleric and isobutyric acid (Yvon & Rijnen, 2001). Non-oxidative enzymatic decarboxylation of α-keto acids to aldehydes is catalyzed in *L. lactis* by an α-ketoisovalerate decarboxylase (De la Plaza, Fernández de Palencia, Peláez, & Requena, 2004). Aldehydes can also be generated by chemical oxidation of α-keto acids catalyzed by bivalent cations (Nierop-Groot & de Bont, 1999; Bonnarme, Amarita, Chambellon, Semon, Spinnler, & Yvon, 2004).

The contribution to cheese aroma of aromatic aldehydes such as phenyl acetaldehyde has been related to a floral note in Camembert (Kubícková & Grosch, 1997). Branched chain aldehydes are a major part of the volatile fraction of several cheeses (Engels, Dekker, de Jong, Neeter, & Visser, 1997). They have been related to malty flavours (Morgan, 1976; Rychlik & Bosset, 2001), but likewise 3-methylbutanal seems to be important to mask the strong aroma of butyric acid in Emmental cheese (Preininger, Warmke, & Grosch, 1996). Alcohols are formed from aldehydes by chemical or enzymatic reactions. As well as aldehydes, they contribute to a good overall flavour when they are in balance with other volatile compounds (Preininger et al., 1996).

Wild lactococcal strains seem to contain more active amino acid converting enzymes than starter strains (Ayad, Verheul, De Jong, Wouters, & Smit, 1999), leading to new aroma descriptors in cheese sensory analysis (Morales, Fernández-García, Gaya, & Núñez, 2003). Combination of wild strains can also contribute to aroma diversification by completion of the flavour formation pathways (Ayad, Verheul, Wouters, & Smit, 2001; Kieronczyk et al., 2003). The aim of the present work has been to evaluate the abilities of a number of wild *L. lactis* strains possessing complementary enzymatic

activities to produce cheese aroma compounds that were evaluated by means of gas chromatography and sensory analyses.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Wild strains of *Lactococcus lactis* IFPL730, IFPL112, IFPL363, IFPL953 and IFPL326 used in this study, were isolated from raw milk cheeses. Working cultures were prepared from a stock maintained at –80 ºC in 10% reconstituted skimmed milk (Scharlau, Barcelona, Spain) by two overnight transfers at 30 °C in M17 broth (Scharlau) supplemented with 0.5% glucose (G-M17).

An overnight culture of each strain was used to inoculate (0.1%) 10 ml G-M17 broth and incubated for 8 h at 30 ºC. Forty millilitres sterile (110 ºC, 10 min) 10% reconstituted skimmed milk powder were inoculated at 2% with each strain and incubated at 30 °C for 24 h. Combination of strains (1:1) with *L. lactis* IFPL 730 were also used to inoculate (2%) skimmed milk. *L. lactis* IFPL730 showing α-keto acid decarboxylase activity (Amárita, Fernández-Esplá, Requena, & Peláez, 2001b; De la Plaza et al., 2004) was used in the mixed cultures to complete the amino acid transamination pathway leading to production of aldehydes.

2.2. Enzymatic assays

The branched-chain and aromatic aminotransferase activities of the lactococcal strains were determined by measuring the formation of α-ketomethylvaleric acid (KMVA) from isoleucine and phenylpyruvic acid (PPA) from phenylalanine, respectively. The reaction mixtures (0.25 ml) contained either L-isoleucine or L-phenylalanine (10 mM) in 70 mM Tris-HCl (pH 7.8), 10 mM α-ketoglutarate, 0.1 mM pyridoxal 5'-phosphate (PLP) and 0.1 mg ml^{-1} cell free extract (CFE), obtained as described by Amarita et al. (2001b). The α-keto acids obtained after 3 h-incubation at 37 ºC were analysed by RP-HPLC using a Jasco RP-HPLC system (Jasco Co., Tokyo, Japan) as previously described (Amárita et al., 2001b).

The glutamate dehydrogenase activity of the strains was determined by measuring the glutamate-dependent reduction of NAD and NADP. The reaction mixture contained 40 mM Tris-HCl buffer (pH 8.8), 1 mM NAD^+ or $NADP^+$, 10mM L-glutamate and 0.1 mg ml^{-1} CFE in a total volume of 1 ml. The amount of NADH or NADPH formed by reduction of the substrate was monitored continuously by measuring absorbance at 340 nm in a Shimadzu UV-1601 spectrophotometer with a thermostatically CPS-240 controller (Shimadzu, Kyoto, Japan).

Enzymatic activities were expressed in µmol of product released per min and mg protein.

2.3. Analysis of volatile compounds by solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS)

Ten millilitres of the incubated milks and 2 g NaCl were added to a 20 ml glass sample vial and fitted with a self-sealing septum at its top, through which the SPME syringe needle (bearing a fibre coated with 85 µm film thickness polyacrylate bonded to

a flexible fused silica core, Supelco, Bellefonte, USA) was introduced and maintained in the head space. The sample was then thermostated at 60 ºC for 40 min. The polar volatile analytes absorbed to the SPME fibre were then analysed by GC–MS (Agilent 6890N Network GC System, Palo Alto, USA). The compounds were separated by onto a HP-INNO-WAX polyethylene glycol capillary column (60 m × 250 µm × 0.5 µm, Agilent). The injection port was heated at 220 ºC, in splitless mode, and He flow was maintained at 1 ml min^{-1}. The SPME fibre was maintained in the injection port for 10 min. The oven temperature programme was held at 45 ºC for 12.5 min, increased to 114 ºC at a rate of 4 ºC min^{-1}, held at 114 ºC for 6 min, then increased to 143 ºC at a rate of 7 ºC min^{-1} and to 240 ºC at 15 ºC min^{-1}, and held at 240 ºC for 4 min. Detection was performed with the mass spectrometer detector (MS 5973N, Agilent) operating in the scan mode (2.81 scan s^{-1}) and the ionization energy set at 70 eV. The temperatures of the ion source and the quadrupole mass analyser were held at 230 ºC and 150 ºC, respectively. The eluted compounds were identified by their retention times and by comparison of their mass spectra with those in the Wiley Mass Spectral database (Wiley & Sons Inc., New York, USA). Relative abundance of compounds was expressed in arbitrary units as peak areas referred to carbinol area, which was employed as internal standard. Samples were analysed in triplicate.

2.4. Sensory analysis

Aroma of the incubated milks was evaluated by 26 panellists. Members were asked for sensory descriptors such as yoghurt, butter, fresh cheese, ripened cheese and aromatic flavour (floral, herbaceous, almond-like or fruity), and the overall aroma

intensity of samples. Descriptors and aroma intensity were scored after sniffing the samples on a scale ranging from 0 (lacking) to 3 (strong).

2.5. Statistical analysis

The StatGraphics plus (version 2.1, Statistical Graphics Corp., Rockville, USA) was used for statistical data processing. Analysis of variance (F-test) was run on the volatile compounds detected by GC-MS and on aroma descriptors to ascertain whether or not the differences in volatile formation between strains were significant ($P < 0.05$). Principal component analysis (PCA) was applied to correlate the abundances of volatile compounds with the scores of aroma descriptors.

3. Results and discussion

Relevant enzymes involved in the amino acid transamination pathway leading to formation of aldehydes are aminotransferases and α-keto acid decarboxylases. The amino group acceptor, α-ketoglutarate, is a limiting factor of the transamination reaction (Yvon et al., 1998) and therefore, the presence of glutamate dehydrogenase activity in LAB can be of key importance for volatiles formation in cheese. In the present work lactococcal strains were evaluated for the presence of aminotransferase and glutamate dehydrogenase activities. Results are shown in Table 1. Aminotransferase activity was present in all strains being higher towards branched chain than aromatic amino acids. Only strains IFPL112 and IFPL363 showed glutamate dehydrogenase activity. *L. lactis* IFPL730 has been described to exhibit α-keto acid

decarboxylase activity with high specificity towards α-ketoisovalerate derived from valine transamination (De la Plaza et al., 2004). The high specificity of the *L. lactis* α-ketoisovalerate decarboxylase entitle it as the rate-controlling step in the formation of branched-chain aldehydes by *L. lactis* (De la Plaza et al., 2004).

Lactococcal strains were combined with *L. lactis* IFPL730 for incubation in skimmed milk in order to complete the transamination metabolic pathway leading to production of aldehydes, and without the need of adding exogenous α-ketoglutarate. The volatile compounds produced by single and mixed cultures after 24 h incubation in skimmed milk were analyzed by solid-phase microextraction (SPME) and evaluated by GC-MS. SPME is a simple and sensitive technique for the concentration of volatile compounds that has been described useful for the characterization of cheese aroma by GC analysis (Lecanu, Ducruet, Jouquand, Gratadoux, & Feigenbaumet, 2002; Frank, Owen, & Patterson, 2004). Main volatile compounds obtained during strains growth in milk are listed in Table 2, which include short chain fatty acids, alcohols, aldehydes and esters. In general, combination of strains with *L. lactis* IFPL730 increased volatile content in the incubated milks, although a direct correlation between presence of glutamate dehydrogenase activity in strains IFPL112 and IFPL363 and volatile compounds increase could not be established. The presence of IFPL730 showing α-keto acid decarboxylase activity in mixed cultures increased acid, alcohol and ester contents, being the highest values found with the combination of strains IFPL730 and IFPL326 (Table 2). Interestingly, *L. lactis* IFPL326 showed the highest aminotransferase activity towards isoleucine (Table 1), which is a specific substrate for the lactococcal branched chain aminotransferase (Atiles et al., 2000; Yvon et al., 2000). The combination of strain IFPL326 with strain IFPL730, which is characterized by the presence of an α-keto acid decarboxylase with high specificity for branched chain α-keto acids (De la

Plaza et al., 2004), contributed to the highest formation in the incubated milk of leucine derived volatile compounds such as 3-methylbutanal, 3-methyl-1-butanol and 2-hydroxy-4-methyl pentanoic acid methyl ester (Table 2). In addition, the milk incubated with the combination of strains IFPL326 and IFPL730, showing the highest content in volatile compounds, was awarded by the test panellists with the highest scores for "ripened cheese" attribute and aroma intensity (Table 3).

All data obtained (volatile compound abundances and aroma descriptor scores) were analyzed by PCA. The study showed that the first six principal components defined the 81.9% of the variability of the system (data not shown). Three-dimensional plot of the three first principal components 1, 2 and 3 (Fig. 1), which accounted for 69.4% of the variability, showed that the sensory attribute “ripened cheese” was well correlated with the presence of 3-methylbutanal, 3-methyl-1-butanol, 2-hydroxy-4-methyl pentanoic acid methyl ester and 2-phenyl ethanol. All four compound contents were highest in the milk incubated with strains IFPL730 and IFPL326 (Table 2). 3-Methyl butanal has been related to cheesy aroma in Gorgonzola cheese (Moio, Piombino, & Addeo, 2000) and 3-methyl-1-butanol has been found to confer aroma of fresh cheese to bovine Mozzarella cheese (Moio, Langlois, Etievant, & Addeo, 1993). In general, it has been considered that hydroxyacid formation produces a leakage in the pathway of aroma compounds formation from keto acids (Yvon & Rijnen, 2001; Van Kranenburg et al., 2002). However, the high abundance of 2-hydroxy-4-methyl pentanoic acid methyl ester in the milk incubated with strains IFPL730 and IFPL326 and its correlation with ripened cheese attribute suggests that α-keto acid reduction is not a closed way for aroma compounds formation.

In the other hand, aromatic aroma descriptors were not well correlated with compounds such as 2-phenyl ethanol, which has been described as floral rose-like

(Kubícková & Grosh, 1997; Roger, Degas, & Gripon, 1988), or 4-ethoxy ethylbenzoate reported as fruity (Mauriello, Moio, Moschetti, Piombino, Addeo, & Coppola., 2001). The milk incubated with strains IFPL730 and IFPL326 also showed the highest abundance of both compounds (Table 2).

The incorporation of *L. lactis* IFPL730 to the incubated milks also caused an increase in the content of short chain fatty acids (Table 2). Above all these compounds, hexanoic acid content greatly increased by the combination of strains IFPL730 and IFPL326. This compound was nearly associated to ripened cheese attribute by PCA (Fig. 1), while butanoic acid was related to butter-like, fruity or yoghurt-like descriptors. Comparing these results and those described in literature, aroma descriptors do not match exactly for the same molecules since both butanoic and hexanoic acids have been related to cheese aroma (Urbach, 1995; Moio & Addeo, 1998). This point is in accordance with the established fact that the perception threshold of a compound is dependent on the matrix and the overall of molecules, and may be responsible of the modification of perceptions for a given compound (Curioni & Bosset, 2002).

In conclusion, combination of *L. lactis* strains harbouring complementary amino acid catabolic routes can contribute to improve cheese aroma formation, resulting the combined cultures with *L. lactis* IFPL730 in higher volatile compound formation in milk than isolate strains. The impact on aroma intensity obtained by the incorporation in the culture of a strain showing α-keto acid decarboxylase activity identifies this as key reaction in the enzymatic conversion of amino acids, therefore being the rate-controlling step in volatile compounds formation. More studies regarding effect of the combined strains in cheese ripening are needed.

Acknowledgements

This work was performed under the auspices of the Consejo Superior de Investigaciones Científicas and was supported by Research Project AGL2002-03277.

References

Amárita, F. Requena, T., Taborda, G., Amigo, L., & Peláez, C. (2001a). *Lactobacillus casei* and *Lactobacillus plantarum* initiate catabolism of methionine by transamination. *Jounal of Applied Microbiology, 90,* 971–978.

Amárita, F., Fernández-Esplá, D., Requena, T., & Pelaez, C. (2001b). Conversion of methionine to methional by *Lactococcus lactis*. *FEMS Microbiology Letters, 204*, 189–195.

Atiles, M. W., Dudley, E., & Steele, J. (2000). Gene cloning, sequencing and inactivation of the branched chain aminotransferase of *Lactococcus lactis* LM0230. *Applied and Environmental Microbiology, 66,* 2325–2329.

Ayad, E. H. E., Verheul, A., De Jong, C., Wouters, J. T. M., & Smit, G. (1999). Flavour forming abilities and amino acid requirements of *Lactococcus lactis* strains isolated from artisanal and non-dairy origin. *International Dairy Journal, 9*, 725–735.

Ayad, E. H. E., Verheul, A., Wouters, J. T. M., & Smit, G. (2001). Population dynamics of lactococci from industrial, artisanal and non-dairy origins in defined strain starters for Gouda-type cheese. *International Dairy Journal, 11*, 51–61.

Banks, J. M., Yvon, M., Gripon, J. C., de la Fuente, M. A., Brechany, E. Y., Williams, A. G., & Muir, D. D. (2001). Enhancement of amino acid catabolism in Cheddar

cheese using alpha-ketoglutarate: amino acid degradation in relation to volatile compounds and aroma character. *International Dairy Journal, 11*, 235–243.

Bonnarme, P., Amarita, F., Chambellon, E., Semon, E., Spinnler, H. E., & Yvon, M. (2004). Methylthioacetaldehyde, a possible intermediate metabolite for the production of volatile sulphur compounds from L-methionine by *Lactococcus lactis*. *FEMS Microbiology Letters, 236,* 85–90.

Curioni, P. M. G., & Bosset, J.O. (2002). Key odorants in various cheese types as determined by gas chromatography-olfactometry. *International Dairy Journal, 12*, 959–984.

De la Plaza, M., Fernández de Palencia, P., Peláez, C., & Requena, T. (2004). Biochemical and molecular characterization of α-ketoisovalerate decarboxylase, an enzyme involved in the formation of aldehydes from amino acids by *Lactococcus lactis*. *FEMS Microbiology Letters, 238,* 365–374.

Dudley, E. G., & Steele, J. L. (2001). *Lactococcus lactis* LM0230 contains a single aminotransferase involved in aspartate biosynthesis, which is essential for growth in milk. *Microbiology, 147,* 215–224.

Engels, W. J. M., Dekker, R., de Jong, C., Neeter, R., & Visser, S. (1997). A comparative study of volatile compounds in the water-soluble fraction of various types of ripened cheese. *International Dairy Journal, 7*, 255–263.

Frank, D. C., Owen, C. M., & Patterson, J. (2004). Solid phase microextraction (SPME) combined with gas-chromatography and olfactometry-mass spectrometry for characterization of cheese aroma compounds. *Lebensmittel Wissenschaft und Technology, 37*, 139–154.

Gao, S., & Steele, J. L. (1998). Purification and characterization of oligomeric species of an aromatic amino acid aminotransferase from *Lactococcus lactis* subsp. *lactis* S3. *Journal of Food Biochemistry, 22*, 197-211.

Kieronczyk, A., Skeie, S., Langsrud, T., & Yvon, M. (2003). Cooperation between *Lactococcus lactis* and non-starter lactobacilli in the formation of cheese aroma from amino acids. *Applied and Environmental Microbiology, 69*, 734–739.

Kubícková, J., & Grosch, W. (1997). Evaluation of potent odorants of Camembert cheese by dilution and concentration techniques. *International Dairy Journal, 7*, 65–70.

Lecanu, L., Ducruet, V., Jouquand, C., Gratadoux, J. J., & Feigenbaum, A. (2002). Optimization of headspace solid-phase microextraction (SPME) for the odor analysis of surface-ripened cheese. *Journal of Agricultural and Food Chemistry, 50*, 3810–3817.

Mauriello, G., Moio, L., Moschetti, G., Piombino, P., Addeo, F., & Coppola, S. (2001). Characterization of lactic acid bacteria strains on the basis of neutral volatile compounds produced in whey. *Journal of Applied Microbiology, 90*, 928–942.

Moio, L., & Addeo, F. (1998). Grana Padano cheese aroma. *Journal of Dairy Research, 65*, 317–333.

Moio, L., Langlois, D., Etievant, P. X., & Addeo, F. (1993). Powerful odorants in water buffalo and bovine Mozzarella cheese by use of extract dilution sniffing analysis. *Italian Journal of Food Science, 3*, 227–237

Moio, L., Piombino, P., & Addeo, F. (2000). Odour-impact compounds of Gorgonzola cheese. *Journal of Dairy Research, 67*, 273–285.

Morales, P., Fernández-García, E., Gaya, P., & Núñez, M. (2003). Formation of volatile compounds by wild *Lactococus lactis* strains isolated from raw ewe´s milk cheese *International Dairy Journal, 13*, 201–209.

Morgan, M. E. (1976). The chemistry of some microbially induced flavour defects in milk and dairy foods. *Biotechnology and Bioengineering, 18*, 953–965.

Nierop-Groot, M. N., & de Bont, J. A. M. (1999). Involvement of manganese in conversion of phenylalanine to benzaldehyde by lactic acid bacteria. *Applied and Environmental Microbiology, 65*, 5590–5593.

Preininger, M., Warmke, R., & Grosch, W. (1996). Identification of the character impact flavour compounds of Swiss cheese by sensory studies of models. *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung, 202*, 30–34.

Roger, S., Degas, C., & Gripon, J. C. (1988). Production of phenyl ethyl alcohol and its esters during ripening of traditional Camembert. *Food Chemistry, 28*, 1–12.

Rychlik, M., & Bosset, J.O. (2001). Flavour and off-flavour compounds of Swiss Gruyère cheese. Evaluation of potent odorants. *International Dairy Journal, 11*, 895–901.

Tanous, C., Kieronczyk, A., Helinck, S., Chambellon, E., & Yvon, M. (2002). Glutamate dehydrogenase activity: a major criterion for the selection of flavour-producing lactic acid bacteria strains. *Antonie van Leewenhoek, 82*, 271–278.

Thage, B. V., Rattray, F. P., Laustsen, M. W., Ardö, Y., Barkholt, V., & Houlberg, U. (2004). Purification and characterization of a branched-chain amino acid aminotransferase from *Lactobacillus paracasei* subsp *paracasei* CHCC 2115. *Journal of Applied Microbiology, 96*, 593–602.

Urbach, G. (1995). Contribution of lactic acid bacteria to flavour compound formation in dairy products. *International Dairy Journal, 5*, 877–903.

Van Kranenburg, R., Kleerebezem, M., Vlieg, J. V., Ursing, B. M., Boekhorst, J., Smit, B. A., Ayad, E. H. E., Smit, G., & Siezen, R. J. (2002). Flavour formation from amino acids by lactic acid bacteria: predictions from genome sequence analysis. *International Dairy Journal, 12*, 111–121.

Williams, A. G., Noble, J., & Banks, J. M. (2001). Catabolism of amino acids by lactic acid bacteria isolated from Cheddar cheese. *International Dairy Journal, 11*, 203–215.

Yvon, M., & Rijnen, L. (2001). Cheese flavour formation by amino acid catabolism. *International Dairy Journal, 11*, 185–201.

Yvon, M., Thirouin, R., Rijnen, L., Fromentier, D., & Gripon, J. C. (1997). An aminotransferase from *Lactococcus lactis* initiates conversion of amino acids to cheese flavour compounds. *Applied and Environmental Microbiology, 63*, 414–419.

Yvon, M., Berthelot, S., & Gripon, J. C. (1998). Adding α-ketoglutarate to semi-hard cheese curd highly enhances the conversion of amino acid to aroma compounds. *International Dairy Journal, 8*, 889–898.

Yvon, M., Chambellon, E., Bolotin, A., & Roudot-Algaron, F. (2000). Characterization and role of the branched-chain aminotransferase (BcaT) isolated from *Lactococcus lactis* subsp. *cremoris* NCDO 763. *Applied and Environmental Microbiology, 66*, 571–577.

Legend to Figure

Fig. 1. Principal component analysis plot (three-dimensional) from volatile compound data and aroma descriptor scores for *Lactococcus lactis* incubated milk. 1: 3-methylbutanal; 2: 3-methyl-1-butanol; 3: 2-hydroxy-4-methyl pentanoic acid methyl ester; 4: 2-phenyl ethanol; 5: 4-ethoxy benzoic acid ethyl ester; 6: hexanoic acid; 7: acetic acid; 8: 2-furanmethanol; 9: octanoic acid; 10: ethanol; 11: benzaldehyde; 12: butanoic acid; A: ripened cheese; B: yoghurt; C: butter; D: fresh cheese; E: herbaceous; F: floral; G: fruity and H: almond-like.

Table 1.

Glutamate dehydrogenase (Gdh), branched-chain aminotransferase (BcaT) and aromatic aminotransferase (AraT) activities of wild *Lactococcus lactis* strains.

	Enzymatic activities[1]		
L. lactis strain	Gdh	BcaT	AraT
IFPL112	0.018	0.140	0.011
IFPL363	0.020	0.159	0.014
IFPL953	ND	0.127	0.094
IFPL326	ND	0.179	0.064
IFPL730	ND	0.126	0.081

[1]Activity expressed as µmol of NADPH (Gdh), KMVA (BcaT) or PPA (AraT) released per min and mg protein. ND: not detected

Table 2.

Relative abundance[1] (mean ± SE) of volatile compounds obtained by solid phase microextraction (SPME) from *Lactococcus lactis* incubated milk.

Compound	*RT*[2]	IFPL730	IFPL112	IFPL363	IFPL953	IFPL326	730-112	730-363	730-953	730-326
3-Methylbutanal	12.71	ND	ND	ND	ND	ND	ND	ND	ND	1.20±0.06a
Etanol	13.89	0.19±0.00a	0.82±0.01cd	0.28±0.04ab	1.04±0.09de	0.53±0.03bc	1.21±0.25e	1.18±0.07e	0.66±0.01c	1.25±0.18e
3-Methyl-1-butanol	29.07	0.18±0.00a	ND	ND	0.83±0.05b	0.46±0.24a	0.22±0.01a	0.27±0.15a	1.10±0.03b	4.59±0.19c
Acetic acid	41.33	0.18±0.01a	0.38±0.01bc	0.13±0.01a	0.28±0.00ab	0.29±0.15ab	0.50±0.09c	0.73±0.04d	0.47±0.03bc	1.02±0.10e
2-Hydroxy-4-methyl pentanoic acid methyl ester	41.95	0.09±0.01a	ND	ND	0.31±0.02bc	ND	0.24±0.02abc	0.16±0.08ab	0.38±0.01c	1.51±0.20d
Benzaldehyde	43.46	0.03±0.02a	ND	0.03±0.02a	0.05±0.03a	0.02±0.02a	0.13±0.01b	ND	0.05±0.05a	ND
4-Ethoxy benzoic acid ethyl ester	44.78	ND	ND	ND	0.77±0.38b	0.06±0.03a	2.83±0.29c	2.59±0.14c	2.55±0.21c	4.60±0.10d
Butanoic acid	45.01	0.08±0.01a	ND	0.06±0.03a	0.57±0.46a	ND	0.51±0.29a	0.10±0.10a	0.39±0.27a	0.14±0.14a
2-Furanmethanol	45.35	0.06±0.03a	0.18±0.01bcd	ND	0.14±0.02abc	0.06±0.06bc	0.21±0.04cd	0.36±0.06ef	0.26±0.04de	0.48±0.06f
Hexanoic acid	47.73	0.39±0.02a	1.03±0.07bcd	0.41±0.07a	0.99±0.05bc	0.80±0.04ab	1.53±0.29cd	1.48±0.04cd	1.56±0.25d	2.35±0.38e
2-Phenyl ethanol	48.88	ND	ND	ND	0.29±0.01ab	0.24±0.00a	0.43±0.01c	ND	0.32±0.01b	0.76±0.07d
Octanoic acid	50.20	0.56±0.03ab	1.49±0.16bcd	0.44±0.05a	0.75±0.38ab	1.06±0.06abc	2.18±0.60d	2.20±0.30d	1.04±0.53ab	1.99±0.10cd

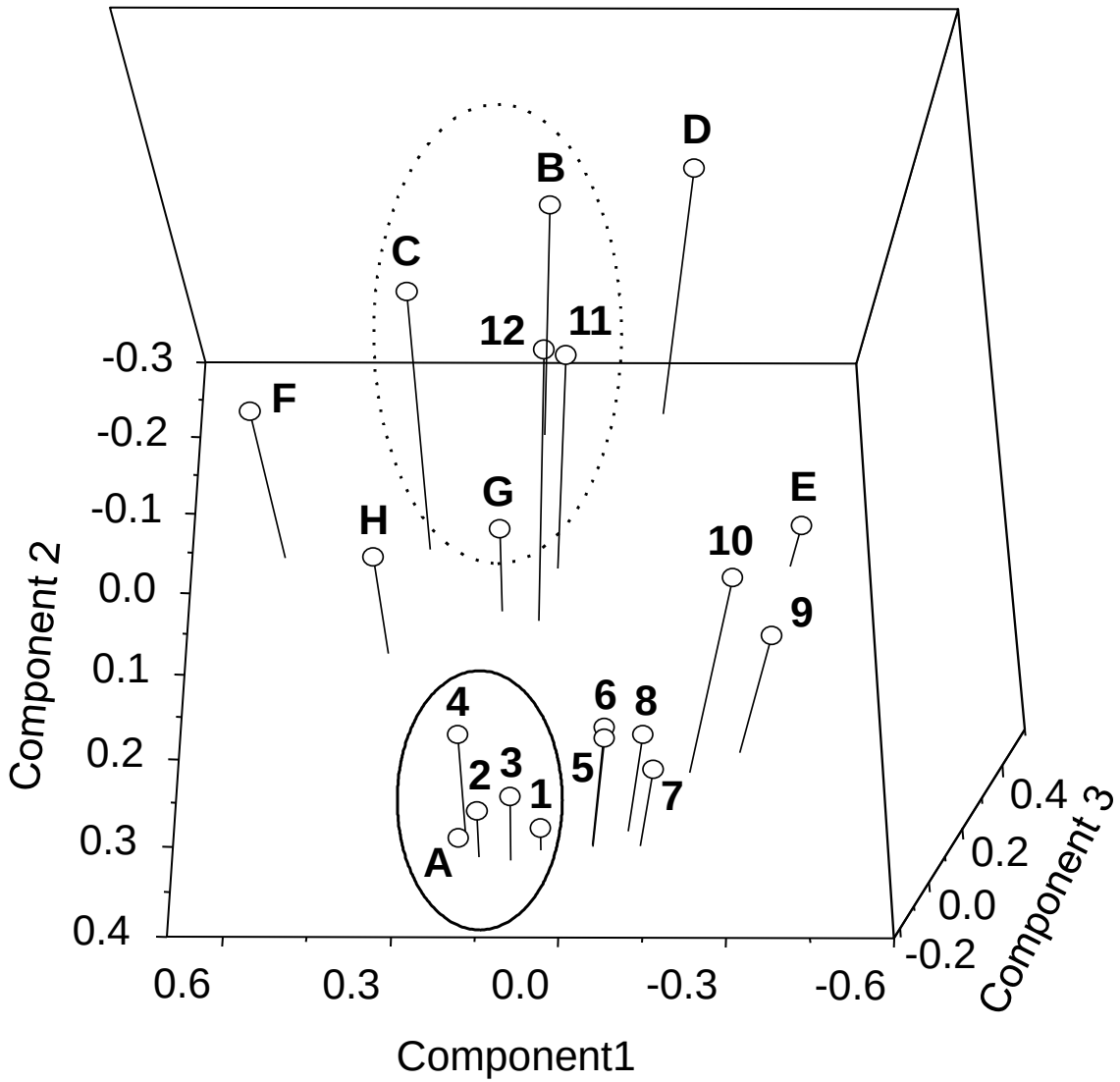
[1]Relative abundance expressed in arbitrary units as percentage of the carbinol peak. ND: not detected. Means followed by the same letter within the same row are not significantly different (P > 0.05).
[2]*RT*, retention time (min).

Table 3.

Aroma scores[1] (mean ± SE) of *Lactococcus lactis* incubated milk.

Aroma descriptor	IFPL730	IFPL112	IFPL363	IFPL953	IFPL326	730-112	730-363	730-953	730-326
Acid	0.23±0.10a	0.31±0.12a	0.35±0.12[a]	0.35±0.12a	0.31±0.12a	0.08±0.05a	0.23±0.10a	0.23±0.10a	0.27±0.12a
Yoghurt	0.50±0.15ab	0.65±0.19b	1.46±0.22c	1.38±0.20c	0.54±0.18ab	0.54±0.17ab	0.88±0.19b	0.73±0.19b	0.15±0.09a
Butter	0.04±0.04a	0.04±0.04a	0.08±0.05[a]	0.35±0.16b	0.15±0.09ab	0.08±0.05a	0.08±0.05a	0.08±0.08a	0.08±0.05a
Fresh cheese	0.15±0.09a	0.15±0.09a	0.19±0.11[a]	0.23±0.14a	0.12±0.06a	0.19±0.10a	0.27±0.12a	0.15±0.09a	ND
Ripened cheese	0.15±0.09a	0.08±0.05a	0.04±0.04[a]	0.23±0.14a	0.27±0.13a	0.08±0.05a	0.04±0.04a	0.27±0.12a	0.85±0.22b
Potato-like	0.19±0.12a	0.12±0.08a	0.12±0.08[a]	0.12±0.08a	0.15±0.12a	0.15±0.12a	0.15±0.09a	0.12±0.12a	0.04±0.04a
Almond-like	0.08±0.08a	0.04±0.04a	0.04±0.04[a]	0.04±0.04a	0.08±0.05a	0.08±0.08a	0.04±0.04a	0.15±0.09a	0.27±0.14a
Fruity	0.08±0.05ab	0.04±0.04a	0.12±0.08[a]b	0.12±0.06ab	0.23±0.13b	0.12±0.08ab	ND	0.04±0.04a	ND
Floral	0.04±0.04a	ND	0.04±0.04[a]	0.08±0.08a	0.08±0.05a	ND	0.15±0.09a	0.12±0.08a	0.15±0.07a
Intensity	1.08±0.12ab	0.88±0.15a	1.35±0.15bc	1.58±0.15c	1.35±0.14bc	1.00±0.14ab	1.04±0.13ab	1.31±0.14bc	2.15±0.16d

[1]Scores vary between 0 (lacking) to 3 (strong). ND: not detected. Means followed by the same letter within the same row are not significantly different ($P > 0.05$).


D
B
C
12
11
F
G
H
E
10
9
4
6
8
5
2
3
1
7
A
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
0.4
Component 2
0.6
0.3
0.0
-0.3
-0.6
Component1
0.4
0.2
0.0
-0.2
Component 3